EXAMPLE 52

NMR experiments on modified oligonucleotides comparison of 3',5' versus 2',5' internucleotide linkages and 2'-substituents versus 3'-substituents by NMR

[0190] The 400MHz ^{1}H spectrum of oligomer d(GAU$_2$*CT), where U$_2$*= 2'-*O*-aminohexyluridine showed 8 signals between 7.5 and 9.0 ppm corresponding to the 8 aromatic protons. In addition, the anomeric proton of U* appears as a doublet at 5.9 ppm with $J_{1',2'}$= 7.5Hz, indicative of C2'-endo sugar puckering. The corresponding 2'-5' linked isomer shows a similar structure with $J_{1',2'}$= 3.85 Hz at 5.75 ppm, indicating an RNA type sugar puckering at the novel modification site favorable for hybridization to an mRNA target. The proton spectrum of the oligomer d(GACU$_3$*), where U$_3$*=3'-*O*-hexylamine, showed the expected 7 aromatic proton signals between 7.5 and 9.0 ppm and the anomeric proton doublet at 5.9 ppm with $J_{1',2'}$= 4.4 Hz. This suggests more of a C3'-endo puckering for the 3'-*O*-alkylamino compounds compared to their 2' analogs. ^{31}P NMR of these oligonucleotides showed the expected 4 and 3 signals from the internucleotide phosphate linkages for d(GAU*CT) and d(GACU*), respectively. 3'-5' Linked vs. 2'-5' linked have different retention times in RP HPLC and hence different lipophilicities, implying potentially different extent of interactions with cell membranes.

EXAMPLE 53

T_m Analysis of 2',5'-linked oligonucleotides versus 3',5'-linked oligonucleotides

[0191] Thermal melts were done as per standarized literature procedures. Oligonucleotide identity is as follows:

Oligonucleotide A is a normal 3'-5' linked phosphodiester oligodeoxyribonucleotide of the sequence d(GGC TGU* CTG CG) SEQ ID NO: 14 where the * indicates the attachment site of a 2'-aminolinker. Oligonucleotide B is a normal 3'-5' linked phosphodiester oligoribonucleotide of the sequence d(GGC TGU* CTG CG) SEQ ID NO: 14 where the * indicates the attachment site of a 2'-aminolinker. Each of the ribonucleotides of the oligonucleotide, except the one bearing the * substituent, are 2'-O-methyl ribonucleotides. Oligonucleotide C has 2'-5' linkage at the * position in addition to a 3'-aminolinker at this site. The remainder of the oligonucleotide is a phosphodiester oligodeoxyribonucleotide of the sequence d(GGC TGU*

CTG CG) SEQ ID NO: 14. The base oligonucleotide (no 2'-aminolinker) was not included in the study.

Table IIIa

OLIGONUCLEOTIDE	MODIFICATION		DNA TARGET	RNA TARGET
A	none		52.2	54.1
	2'-aminolinker	50.9		55.5
B	none		51.5	72.3
	2'-aminolinker	49.8		69.3
C	none		NA	
	3'-aminolinker	42.7		51.7

[0192] The 2'-5' linkages demonstrated a higher melting temperature against an RNA target compared to a DNA target.

EXAMPLE 54

Snake Venom Phosphodiesterase and Liver Homogenate Experiments on Oligonucleotide Stability

[0193] The following oligonucleotides were synthesized following the procedure of Example 49.

Table IV
Modified Oligonucleotides
synthesized to evaluate stability

SEQ ID NO. #	(ISIS)#	Sequence (5'-3')	Backbone	Chemistry
15	(22110)	TTT-TTT-TTT-TTT-TTT-**T*T*T*-T***	P=O	3'-*O*-MOE
16	(22111)	TTT-TTT-TTT-TTT-TTT-**T#T#T#-U#**	P=O	3'-*O*-MOE
15	(22112)	TTT-TTT-TTT-TTT-TTT-**T*T*T*-T***	P=S	3'-*O*-MOE
16	(22113)	TTT-TTT-TTT-TTT-TTT-**T#T#T#-U#**	P=S	3'-*O*-MOE

15	(22114)	TTT-TTT-TTT-TTT-TTT$_O$-T*$_O$T*$_O$T*$_O$T*	P=S/P=O	3'-*O*-MOE
16	(22115)	TTT-TTT-TTT-TTT-TTT$_O$-T#$_O$T#$_O$T#$_O$-U#	P=S/P=O	3'-*O*-MOE

[0194] [1]All nucleosides with an asterisk contain 3'-*O*-(2-methoxyethyl). All nucleosides with a # contain 2'-*O*-(2-methoxyethyl).

The oligonucleotides were purified following the procedure of Example 50 and analyzed for their structure.

Table V

Properties of Modified Oligonucleotides

SEQ ID NO. #	(ISIS)#	#Sequence (5'-3')[1]	Expected Mass	Observed Mass	HPLC[2] T_R	#Ods(260nm) Purified (min.)
15	(22110)	TTT-TTT-TTT-TTT-TTT-T*T*T*-T*	6314.189	6315.880	20.39	174
16	(22111)	TTT-TTT-TTT-TTT-TTT-T#T#T#-U#	6004.777	5997.490	20.89	147
15	(22112)	TTT-TTT-TTT-TTT-TTT-T*T*T*-T*	6298.799	6301.730	25.92	224
16	(22113)	TTT-TTT-TTT-TTT-TTT-T#T#T#-U#	6288.745	6286.940	24.77	209
15	(22114)	TTT-TTT-TTT-TTT-TTT$_O$-T*$_O$T*$_O$T*$_O$T*	6234.799	6237.150	24.84	196
16	(22115)	TTT-TTT-TTT-TTT-TTT$_O$-T#$_O$T#$_O$T#$_O$-U#	6224.745	6223.780	23.30	340

[0195] [1]All nucleosides with an asterisk contain 3'-*O*-(2-methoxyethyl). All nucleosides with a # contain 2'-*O*-(2-methoxy) ethyl. [2]Conditions: Waters 600E with detector 991; Waters C4 column (3.9X300mm); Solvent A: 50 mM TEA-Ac, pH 7.0; B: 100% acetonitrile; 1.5 mL/min. flow rate; Gradient: 5% B for first five minutes with linear increase in B to 60% during the next 55 minutes.

EXAMPLE 55

3'-O-Aminopropyl modified oligonucleotides

[0196] Following the procedures illustrated above for the synthesis of oligonucleotides, modified 3'-amidites were used in addition to conventional amidites to prepare the oligonucleotides listed in tables VI and VII. Nucleosides used include: N6-benzoyl-3'-O-propylphthalimido-A-2'-amidite, 2'-O-propylphthaloyl-A-3'-amidite, 2'-O-methoxyethyl-